PURIFIED STAT PROTEINS AND METHODS OF PURIFYING THEREOF

GOVERNMENTAL SUPPORT

The research leading to the present invention was supported, at least in part, by NIH Grant Nos. AI32489 and AI34420. Accordingly, the Government may have certain rights in the invention.

CROSS-REFERENCE TO RELATED APPLICATIONS

The present Application is based upon provisional application U.S. Serial No. 60/028,176, filed October 15, 1996, the disclosure of which is hereby incorporated by reference in its entirety. Applicants claim the benefits of this Application under 35 U.S.C. § 119(e).

FIELD OF THE INVENTION

The present invention relates generally to methods of purifying recombinant Stat proteins, modified Stat proteins and functional fragments thereof. Included in the present invention are the purified proteins and fragments themselves. The present invention also relates to methods of separating phosphorylated species of these proteins and fragments from the nonphosphorylated forms. The present invention also relates to methods for using purified Stat proteins, truncated Stat proteins or N-terminal fragments of Stat proteins for drug discovery.

BACKGROUND OF THE INVENTION

Transcription factors play a major role in cellular function by inducing the transcription of specific mRNAs. Transcription factors, in turn, are controlled by distinct signalling molecules. One particular family of transcription factor consists of the Signal Transducers and Activators of Transcription (Stat) proteins. Presently, there are seven known mammalian Stat family members. The recent discovery of Drosophila and Dictyostelium discoideum Stat proteins suggest that Stat proteins have played an important role in signal transduction since the early stages of our evolution [Yan R. *et al.*, *Cell* **84**:421-430 (1996); Kawata *et al.*, *Cell* **89**:909 (1997)].

Stat proteins mediate the action of a large group of signalling molecules including the

cytokines and growth factors (Darnell *et al.* WO 95/08629, 1995). One distinctive characteristic of the Stat proteins are their apparent lack of requirement for changes in second messenger, *e.g.*, cAMP or Ca^{++}, concentrations. Another characteristic is that Stat proteins are activated in the cell cytoplasm by phosphorylation on a single tyrosine (Darnell *et al.*, 1994; Schindler and Darnell, 1995). The responsible kinases are either ligand-activated transmembrane receptors with intrinsic tyrosine kinase activity, such as EGF- or PDGF-receptors, or cytokine receptors that lack intrinsic kinase activity but have associated JAK kinases, such as those for interferons and interleukins (Ihle, 1995). When Stat proteins are phosphorylated, they form homo- or heterodimeric structures in which the phosphotyrosine of one partner binds to the SRC homology domain (SH2) of the other. The newly formed dimer then translocates to the nucleus, binds to a palindromic GAS sequence, thereby activating transcription (Shuai *et al.*, 1994; Qureshi *et al.*, 1995; Leung *et al.*, 1996).

Stat proteins serve in the capacity as a direct messengers between the cytokine or growth factor receptor present on the cell surface, and the cell nucleus. However, since each cytokine and growth factor produce a specific cellular effect by activating a distinct set of genes, the means in which such a limited number of Stat proteins mediate this result remains a mystery. Indeed, at least thirty different ligand-receptor complexes signal the nucleus through the seven known mammalian Stat proteins [Darnell *et al.*, *Science* **277**:1630-1635 (1997)].

Clearly there is a need to further study the biochemistry of Stat proteins. Unfortunately current studies are seriously hampered due to the low quantities of purified protein available. Full-length cDNAs for all mammalian Stats have been cloned. In addition, certain Stat proteins have been expressed in baculovirus-infected insect cells using a His tag at the COOH-terminal end and then purified by Ni-affinity chromatography (Xu, X., *et al.*, *note* 9 (1996). However, no one has reported the production of milligram quantities of activated Stat protein, nor more importantly, a purification process amenable to scaling up for such quantitative isolations.

To perform the biochemical studies necessary to understand the mechanism of the Stat-mediated signal transduction, and to configure assays useful for the detection of compounds that modulate Stat function, there remains an unfulfilled requirement for the